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direct interactions with either the lipid bilayer or other proteins. Upon membrane binding, the N-terminus of α-synuclein forms a helical structure and inserts into the hydrophobic region of the outer membrane leaflet. However, membrane structural changes induced by α-synuclein are still largely unclear. Here we report a substantial area expansion (around 25nm2) induced by the binding of monomer α-synuclein. This measurement is accomplished by observing the increase of membrane area during the binding of α-synuclein with pipette-aspirated giant vesicles. The extent of membrane area expansion is observed to correlate linearly with the density of α-synuclein on the membrane, revealing a constant area increase induced by the binding per α-synuclein molecule. The area expansion per synuclein is found to be independent of aspiration pressure and vesicle size, but exhibits a strong dependence on lipid composition and an up to four-fold decrease with bulk protein concentration. Fragmentation or tubulation of the membrane follows the membrane expansion process; however, no distinct tubulation-transition density, such as observed for BAR domain proteins, can apparently be identified for α-synuclein, suggesting a more complex membrane curvature generation mechanism. Compared with other proteins with membrane insertion capabilities such as ENTH and endophilin N-BAR domains, the linear membrane expansion behaviour is found to be a unique feature for α-synuclein. With measurements of α-synuclein membrane binding energy and membrane physical properties, we hypothesize that the membrane expansion by α-synuclein is the result of α-synuclein induced local thinning of the membrane.

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Acetylation Regulates the Interaction of Huntingtin with Lipid Membranes: Implications for Huntington Disease

Maxmore Chaibva, James R. Arndt, Stephen J. Valentine, Justin Legleiter.
Chemistry, West Virginia University, Morgantown, WV, USA.

Huntington disease (HD) is a genetic neurodegenerative disorder caused by an expanded polyglutamine (polyQ) domain in the N-terminus of the huntingtin (htt) protein which facilitates its aggregation. The first 17 amino acids (Nt17) in htt is an amphiphathic α-helix lipid-binding domain that promotes the formation of a diverse population of nanoscale aggregates. This domain undergoes numerous posttranslational modifications that modulate htt's toxicity, subcellular localization, and trafficking of vesicles. More specifically, N-terminal acetylation of htt has been implicated in the etiology of HD. Given the importance of acetylation in HD, we employed mass spectrometry (MS), both in situ and ex situ atomic force microscopy (AFM), and spectroscopic techniques to evaluate the impact of lysine acetylation on htt's aggregation kinetics in solution and on model lipid bilayers. Acetylation of htt exon 1 (51Q), and synthetic truncated htt exon 1 peptide (Nt17Q35P10KK) was achieved using a selective covalent label sulfo-N-hydroxysuccinimide (NHSA) in molar ratios of 1x, 2x, and 3x NHSA per peptide. With these molar ratios, all three lysine residues (K6, K9, and K15) in Nt17 were significantly labeled, as verified by MS. N-terminal htt acetylation retarded fibril formation in solution; however, the resulting fibril morphology was unaltered. Htt acetylation strongly impacted the protein's ability to bind lipid membranes, as demonstrated by a combination of lipid binding assays and AFM. Acetylated htt was found to bind to lipid vesicles, and disrupt lipid bilayer morphology less aggressively compared to the unlabeled htt. Our results highlight that N-terminal acetylation influences the aggregation of htt and its interaction with lipid bilayers.

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Cholesterol Modulates the Binding and Subsequent Aggregation of Huntingtin on Lipid Bilayers

Xiang Gao, Maxmore Chaibva, Pranav Jain, Justin Legleiter.
Chemistry, West Virginia University, MORGANTOWN, WV, USA.

Huntington disease (HD) is an autosomal dominant inherited neurodegenerative disease caused by abnormally long CAG-repeats in the huntingtin gene. This mutation encodes an expanded polyglutamine (polyQ) domain in the N-terminus of the huntingtin (htt) protein which directly leads to its disease-related aggregation. Htt is found highly associated with a variety of cellular and subcellular membranes, which are predominately comprised of lipids. The interaction of htt with lipid membranes is facilitated by its first 17 amino acids, whose secondary structure is an amphipathic α- helix. There are alterations in the relative amounts of specific membrane components in the brains of HD patients, and in particular, cholesterol homeostasis is altered. Here, we investigate how cholesterol modifies the interaction of htt with lipid bilayers. Using atomic force microscopy (AFM), we track aggregation of htt on supported lipid bilayer containing varying amounts of exogenously added cholesterol. As the amount of cholesterol in the bilayer increased, htt binding to the membrane, and subsequent aggregation, was reduced. This reduced lipid-membrane interaction was further validated using a colorimetric polydiacetylene (PDA) lipid binding assay. More interestingly, morphological changes on the bilayer induced by exposure to htt are significantly altered upon addition of cholesterol.

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Sequence-Independent SSDNA Relieves Phospholamban Inhibition of SERCA in a Length Dependent Manner

Kailey J. Soller[1], Raffaello Verardi[1], Vitaly V. Vostrikov[1], Neha Abrol[2], Seth L. Robia[2], Michael T. Bowser[1], Gianluigi Veglia[1].
[1]Chemistry, University of Minnesota, Minneapolis, MN, USA, [2]Department of Cell and Molecular Physiology, Loyola University Chicago, Maywood, IL, USA.

Muscle contractility is regulated by a network of many proteins. In cardiomyocytes, the sarco(endo)plasmic reticulum Ca^{2+} -ATPase, SERCA, and its regulatory protein, phospholamban are responsible for ~70% of Ca^{2+} reuptake into the SR. While unphosphorylated, PLN inhibits SERCA by lowering its apparent Ca^{2+} affinity. Upon phosphorylation by PKA at Ser16, PLN inhibition is relieved. This tightly regulated interaction can be easily disrupted by mutation or changes in protein level, leading to heart disease. Thus, understanding the molecular interactions between SERCA/PLN and possible regulators is essential.

Here, we report that ssDNA binds the cytoplasmic domain of PLN with low nanomolar dissociation constants, relieving inhibition of SERCA. The relief of inhibition is length dependent, while affinity is constant for oligonucleotides longer than 10 bases. Solution and solid-state NMR experiments have provided residue specific information that ssDNA targets the cytoplasmic domain of PLN and does not affect SERCA in the absence of PLN. In-cell FRET, and NMR experiments determined that addition of ssDNA does not dissociate PLN from SERCA.

SERCA/PLN has become a highly targeted complex for development of small molecule regulators because of its prevalence in many cardiovascular diseases. While some therapies are currently being investigated, none have proceeded past clinical trials. These results provide a promising avenue for development of novel regulators of the SERCA/PLN complex. Additionally, they support previous findings from our group detailing the intricate balance that is necessary for proper cardiac function.

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Systematic Perturbations of Micelle Properties to Investigate the Stabilization of Membrane Protein Structure and Function

Ashton Brock, Shelby Lipes, Linda Columbus.
Chemistry, University of Virginia, Charlottesville, VA, USA.

Membrane protein research is hampered by the difficulty in selecting a membrane mimic that solubilizes and stabilizes protein fold and function. While detergents are often utilized, identifying the appropriate detergent composition to maintain protein solubilization and stability is an expensive, time-consuming, empirical process that is often unsuccessful. The goal of this research is to understand the interactions between detergent and protein by relating physical surfactant properties with membrane protein fold, function, and stability, enabling rational detergent selection. To determine important micelle and protein characteristics, outer membrane phospholipase A1 (OMPLA), the protease OmpT, and the lipid A palmitoyltransferase PagP were purified in different detergent micelles with varying properties such as alkyl chain length, charge, and head group. The overall protein structure and function were evaluated in many pure micelles to identify trends with detergent characteristics and protein function. Upon determination of kinetic parameters for several β-barrel proteins in pure micelles, protein function and structure will be investigated with mixed micelles, to test hypotheses generated by the trends observed in pure micelles. This research will provide a logical rationale for the selection of detergents based on the physical properties of membrane proteins and detergents.

1288-Pos Board B239

Modified Aminophospholipids Strongly Alter the Function of Mitochondrial Membrane Protein UCP1

Olga Jovanovic, Nadine Burchardt, Lars Gille, Elena E. Pohl.
Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria.

Oxidative stress and lipid peroxidation of cell membrane phospholipids are related to many pathological states such as metabolic, cardiovascular and inflammatory diseases. Peroxidation of long chain fatty acids induced by the reactive oxygen species, leads to formation of reactive aldehydes (RA) even